first page picture ~~ghosts~~ Back of under Coal
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A Dynamic Model of Cell Membranes

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The envelopes that surround entire cells, cell nuclei and the various cell organelles are thin assemblies of lipid and protein molecules. Their functions depend on how the membrane proteins are linked

under accumulators

R/B P/S

by Roderick A. Capaldi

The fundamental unit of living tissue is the cell. In recent years it has become plain that one cellular component, the membrane, plays a crucial role in almost all cellular activity. Cytoplasmic membrane, the outer envelope surrounding the cell, acts to regulate the internal environment of the cell and to transport substances into and out of it. Internal membranes, which enclose the nucleus of the cell and such cell organelles as microsomes, mitochondria and the chloroplasts of plants, play an equally important role. For example, the mitochondrial membrane is where adenosine triphosphate (ATP) is manufactured; hence this membrane supplies the fuel for all the cell's metabolic processes. Similarly, the chloroplast membrane is the site of photosynthesis, the process by which energy from the sun is trapped in a form that can be used by living cells. How then are membranes built to accomplish so many different tasks?

A good deal of information now exists concerning the basic structure of membranes. One fact to emerge recently is that cytoplasmic and internal membranes are essentially alike; both are composed of proteins and the fatty substances called lipids. In mammalian cells small amounts of carbohydrate are also present, associated either with protein as glycoproteins, that is, carbohydrate-bearing proteins, or with lipid as glycolipids.

Lipids account for about half of the mass of most membranes. In internal membranes the lipid is almost exclusively phospholipid. Cytoplasmic membranes, in contrast, contain both glycolipid and neutral, or uncharged, lipid in addition to phospholipid. For example, as much as 30 percent of the lipid in the membrane of red blood cells consists of

Individual lipid molecules have a head and two tails [*see illustration on next page*]. At the point where the head and the tails meet, which C. Fred Fox of the University of California at Los Angeles calls the backbone, is a glycerol group. The tails that descend from the backbone are extended chains of fatty acids. The structure of these chains is quite similar to that of oil molecules and, just as oil and water tend to separate into different layers when they are mixed, so do the tails of phospholipid molecules tend to point away from water. Hence they are said to be hydrophobic. The heads of the phospholipid molecules, on the other hand, are soluble in water and are said to be hydrophilic. Molecules of this kind, with one hydrophobic end and one hydrophilic, are called amphipathic. Glycolipids and to some extent neutral lipids are also amphipathic.

The lipids in membranes are arranged so as to accommodate their amphipathic character. They form a bilayer, two layers back to back, so that their hydrophilic heads constitute the top and bottom surfaces of the membrane and their hydrophobic tails are buried in the membrane interior [*see upper illustration on page 29*]. The lipid bilayer is a sheet about 45 angstroms thick. It is the structural framework of the membrane. It is also the anchorage for the other major component of membranes: protein.

Proteins and glycoproteins play a variety of roles in membranes. They can contribute to the structural integrity of the membrane, they can act as enzymes or they can function as pumps, moving material into and out of cells and organelles. It is the diversity of its protein activity that gives each particular membrane its distinctive character.

which membranes can be determined in various ways. One is to assay the membrane's various enzymic activities. Another is to identify proteins by molecular weight, utilizing the technique of gel electrophoresis. Proteins are made up of the long chains of amino acids called polypeptides. Some proteins have only a single polypeptide chain; others have many polypeptide chains tightly associated with one another. In preparation for gel electrophoresis a protein is broken down into its component polypeptide chains by exposure to a detergent, sodium dodecyl sulfate. The chains are then transferred to a polyacrylamide gel with an electric potential across it. They migrate through the gel in response to the potential at a rate proportional to their molecular weight; the lower the weight of the polypeptide, the farther it moves. The gel is then stained with a protein-specific substance, for example coomassie brilliant blue, and is scanned for absorbance. When, for example, the proteins associated with the membrane of the red blood cell are identified in this fashion, the scanning trace reveals polypeptides with molecular weights ranging from 255,000 to 12,500.

The two heaviest polypeptide components, with molecular weights of 255,000 and 220,000, are collectively known as spectrin. (Vincent T. Marchesi of the Yale University School of Medicine chose the name because he first isolated the components from "ghosts," the membranes of red blood cells that have been chemically deprived of their hemoglobin.) Spectrin accounts for about a third of all the protein in the red-cell membrane. Another third of the protein lies in a diffuse absorption band with a molecular weight of about 90,000. This band contains a number of different polypeptides, including a component

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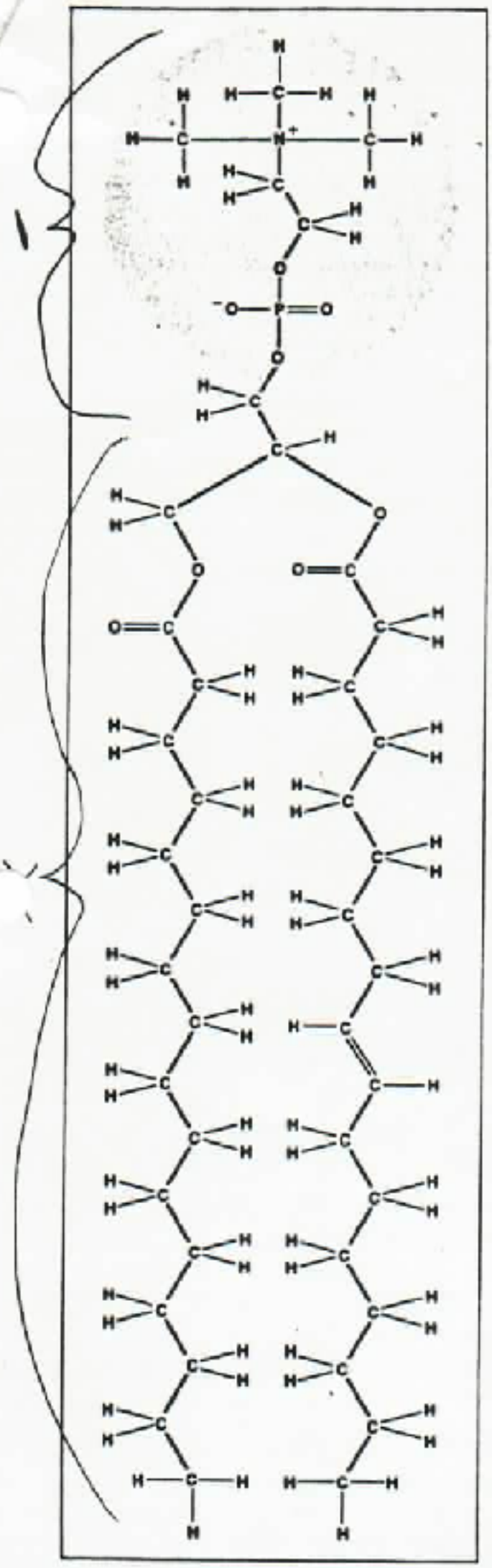


AMPHIPATHIC STRUCTURE of a lipid molecule, with a hydrophilic head and twin hydrophobic tails, is exemplified by this typical phospholipid, specifically a molecule of phosphatidylcholine. Various lipid molecules comprise about half of the mass of mammalian membrane, forming the membrane's structural framework. Their fatty-acid tails may be saturated (*left*), with a hydrogen atom linked to every carbon bond, or unsaturated (*right*), with carbons free.

ent in equal copies with each spectrin molecule. Proteins of molecular weight lower than 70,000 make up the remaining third of the protein in the membrane. The red-cell membrane may be unusual in the large amount of protein of high molecular weight that it incorporates. By way of comparison, almost all the polypeptides in mitochondrial membrane are below 70,000 in weight.

Membrane proteins can be divided into two classes depending on their location with respect to the lipids of the membrane framework. One class consists of those protein molecules that are associated only with the membrane surface. These "extrinsic" proteins are located adjacent to either the outer or the inner surface of the membrane. The second class is made up of proteins that actually penetrate the membrane surface. These "intrinsic" proteins enter the lipid bilayer and sometimes extend all the way through it [*see upper illustration on opposite page*].

Whether a membrane protein should be assigned to one or the other of the two classes can be determined on the basis of its chemical properties or on the basis of other kinds of analysis, such as X-ray diffraction or electron microscopy. For example, extrinsic proteins are relatively easy to remove from membranes by chemical dissociation methods, whereas intrinsic proteins form an integral part of the membrane continuum and are much more difficult to dislodge.

Two extrinsic proteins that are visible in electron micrographs are the enzyme ATPase, found in mitochondrial membrane, and spectrin, the polypeptide in red-cell membrane. Objects termed "headpieces" are visible sticking up from the membrane surface in electron micrographs of mitochondrial membrane; they are ATPase molecules. Similarly, in electron micrographs of red-cell ghosts the "fuzz" lining the inside of the membrane is composed of spectrin polypeptides.

One intrinsic protein that has been studied in detail is rhodopsin, the only protein present in the membranes of the disks that occupy the outer segments of the rod cells of the retina. J. Kent Blasie and his colleagues at the University of Pennsylvania School of Medicine, working with X-ray-diffraction techniques, have found that the rhodopsin molecule is globular and some 42 angstroms in diameter. When the retinal rods are in darkness, the rhodopsin molecules of the disk membrane are submerged for about a third of their diameter in the membrane's outer surface. When the rods are illuminated, the rhodopsin molecules sink deeper into the membrane until they are about half-submerged. Even then, however, the molecules have penetrated less than halfway through the bilayer.

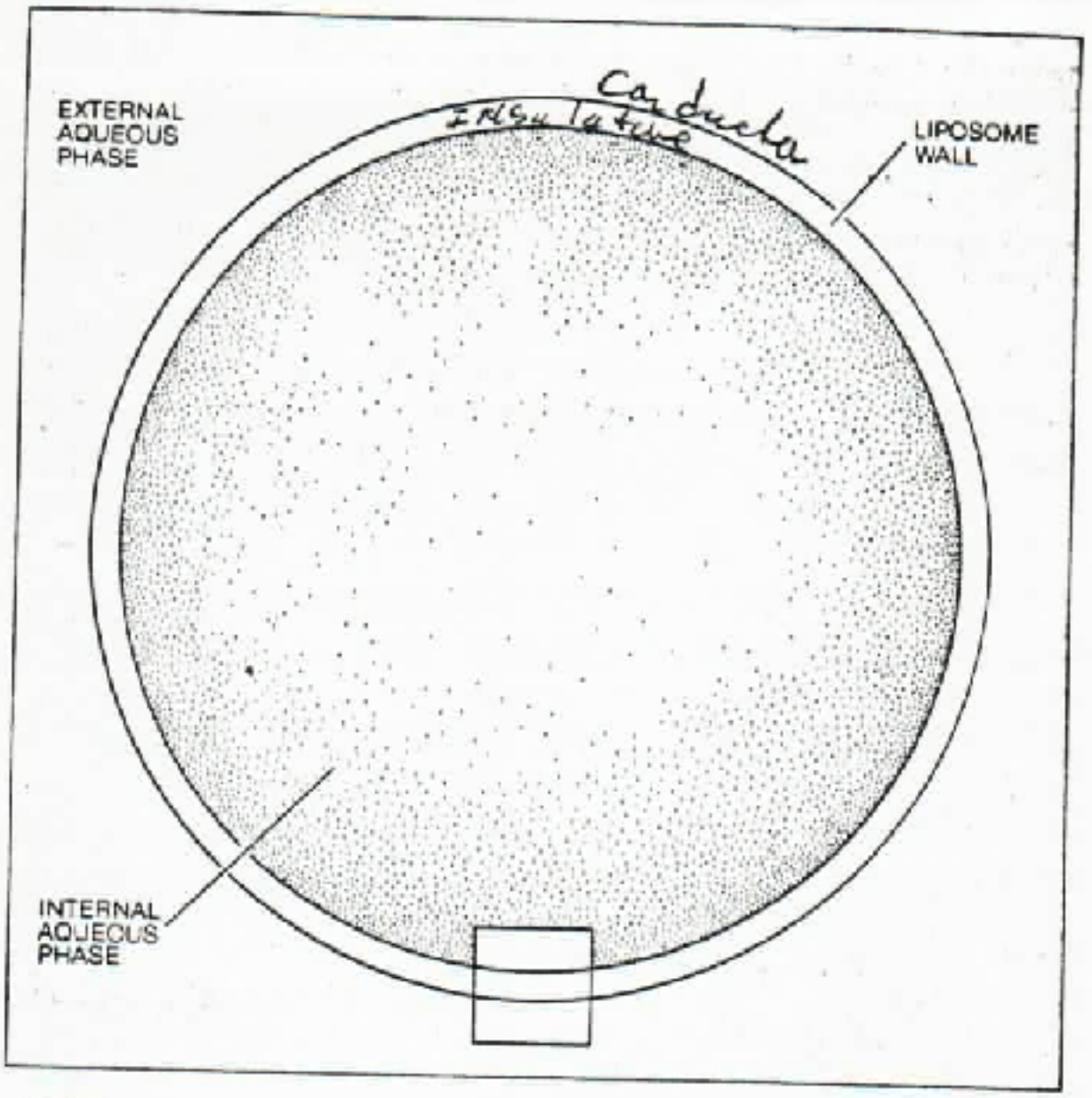
Working in David E. Green's laboratory at the Institute for Enzyme Research at the University of Wisconsin, my colleagues and I have examined the organization of another intrinsic protein: cytochrome oxidase, an enzyme in mitochondrial membrane that is the terminal member of the electron-transfer chain involved in the synthesis of ATP. Now, one stumbling block in the path of membrane-protein research is the fact that most membranes contain a heterogeneous mixture of proteins, including both extrinsic and intrinsic proteins. Most of the methods that can be harnessed to examine the structure of membranes, such as X-ray diffraction, are averaging methods; the resulting data give only the average properties of all the proteins in the sample, whereas we really want to know the characteristics of individual membrane proteins. This is one reason why retinal-disk membrane, with its single protein rhodopsin, is a popular subject of investigation.

Fortunately for us it is possible to separate cytochrome oxidase from the other proteins in mitochondrial membrane. When the separated enzyme is placed in suspension with lipids, the lipids and the enzyme interact and gather in saclike vesicles that are in effect man-made membranes. The molecules of cytochrome oxidase in the artificial vesicles have the same enzymic properties that they show in normal mitochondrial membrane, and so it seems a good bet that the vesicles have the same structure as normal membrane. The advantage here is that a heterogeneous array of proteins has been reduced to a single protein.

We have used these membrane vesicles as a model system for the study of the isolated protein in its geometrical relations with the lipid bilayer. In its capacity as an electron-transfer substance cytochrome oxidase exists in one of two states, either oxidized or reduced. In the oxidized state (and in a narrow range of lipid-to-protein ratios) the enzyme is organized into a crystalline lattice that is visible in the electron microscope and can also be analyzed by X-ray diffraction [*see illustration on page 26*]. Utilizing both kinds of data, we found that individual molecules of cytochrome oxidase are some 55 angstroms long, 60 angstroms wide and 80 to 85 angstroms deep. This is enough depth to allow the molecule to penetrate the 45-angstrom bilayer completely, leaving one end jut-

bacterium *Escherichia coli*. Thus differences in amino acid composition cannot account for the water-insolubility of membrane proteins.

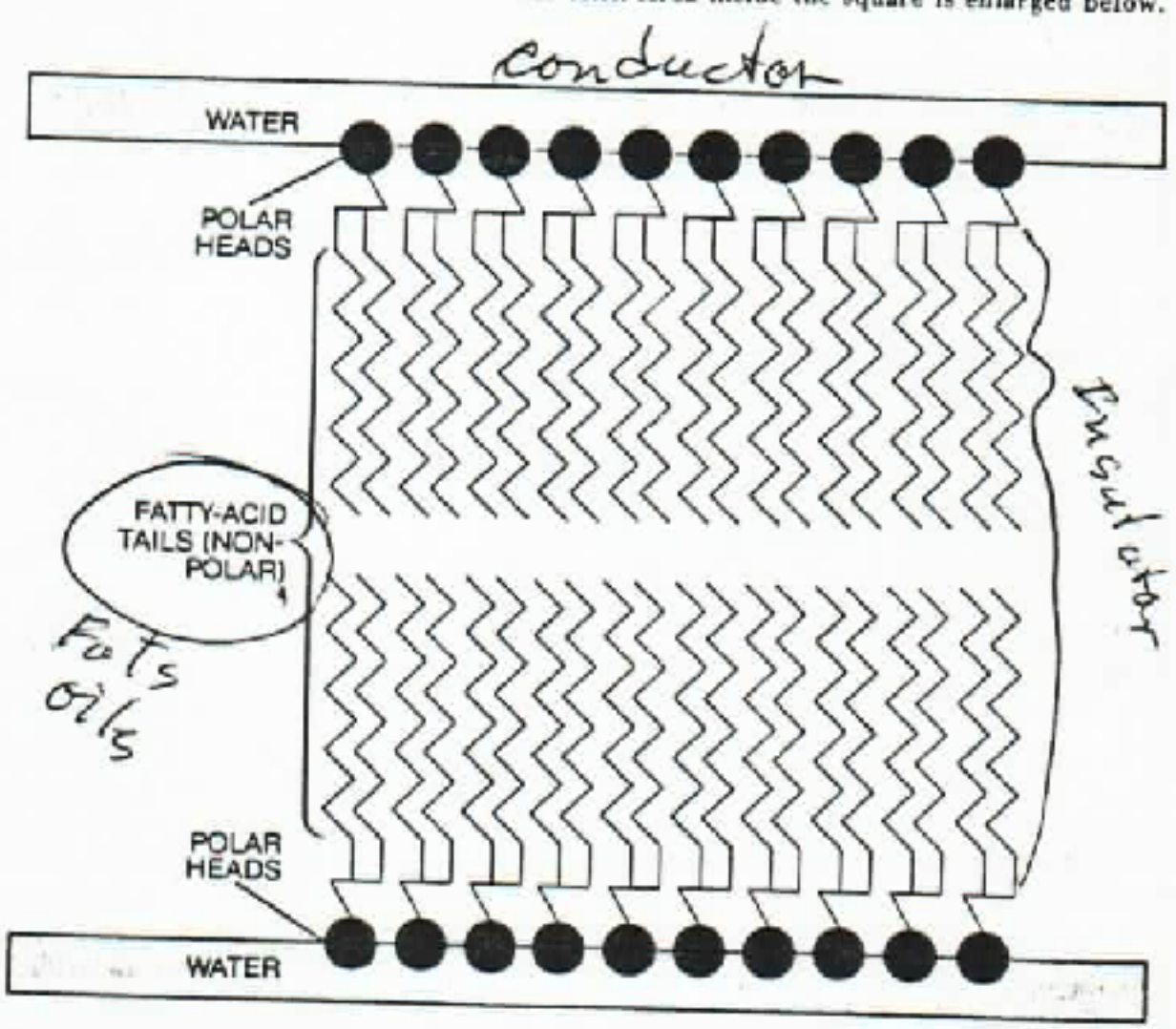
Studies conducted by L. Spatz and Philipp Strittmatter of the University of Connecticut indicate that the most likely explanation for the water-insolubility of membrane proteins is the arrangement of their amino acids. Spatz and Strittmatter subjected membranes of rabbit liver cells to a mild treatment with a proteolytic enzyme. The treatment released the biologically active portion of the membrane protein cytochrome b_5. In a separate procedure they solubilized and purified the intact cytochrome b_5 and treated it with the proteolytic enzyme. This treatment also released the water-soluble, biologically active portion of the molecule, together with a number of small degradation products that were insoluble in aqueous solution. The biologically active portion of the molecule, whether obtained from the membrane or from the purified protein, was found to be rich in polar amino acids. The protein fragments that were insoluble in water, on the other hand, were rich in nonpolar amino acids. These observations suggest that many membrane proteins may be amphipathic, having a nonpolar region that is embedded in the part of the membrane containing the nonpolar fatty-acid tails of the phospholipids and a polar region that is exposed on the membrane surface.



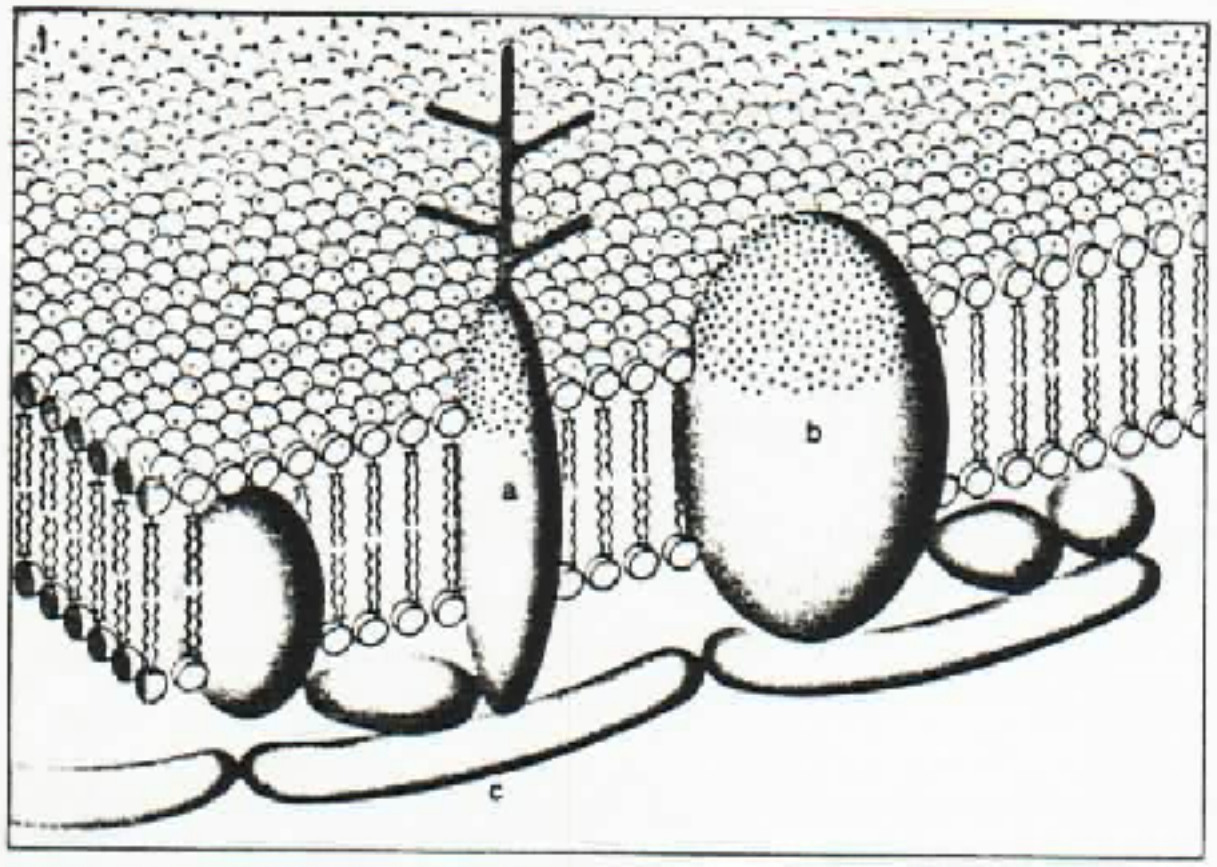
ARTIFICIAL MEMBRANE-ENCLOSED SAC, known as a liposome, is created by subjecting an aqueous suspension of phospholipids to high-energy sound waves. X-ray diffraction shows that the phospholipids in the liposome assume an orderly arrangement resembling what is found in the membranes of actual cells. Area inside the square is enlarged below.

We are now ready to ask: How do substances pass through membranes? The nonpolar fatty-acid-tail region of a phospholipid bilayer is physically incompatible with small water-soluble substances, such as metal ions, sugars and amino acids, and thus acts as a barrier through which they cannot flow freely. If one measures the rate at which blood sugar (glucose) passes through the phospholipid-bilayer walls of liposomes, one finds that it is far too low to account for the rate at which glucose penetrates biological membranes. Information of this kind has given rise to the concept that entities termed carriers must be present in biological membranes to facilitate the passage of metal ions and small polar molecules through the barrier presented by the phospholipid bilayer.

Experiments with biological membranes indicate that the hypothetical carriers are highly selective. For example, a carrier that facilitates the transport of glucose through a membrane plays no role in the transport of amino acids or other sugars. An interesting experimental



CROSS SECTION OF LIPOSOME WALL shows how the membrane is formed from two layers of lipid molecules. The polar heads of amphipathic lipids face toward the aqueous solution on each side while the nonpolar fatty-acid tails face inward toward one another.



ASYMMETRIC DISTRIBUTION of the protein molecules associated with red-blood-cell membrane is demonstrated by labeling the proteins. When a chemical reagent that cannot pass through the lipid bilayer is applied to the cell surface (*1*), only two penetrating proteins with ends that extend above the surface (*a*, *b*) are labeled. When a lysing agent is added, making the membrane leaky (*2*), all the molecules on or near the other side of the membrane are labeled, including the spectrin (*c*) that probably holds the others in place.

trinsic protein of the inner membranes of mitochondria with a molecular weight of 10,000. Its amino acid content is 20 percent hydrophilic and 80 percent hydrophobic. This is in sharp contrast to cytoplasmic and extrinsic membrane proteins, which have on the average 47 percent hydrophilic amino acids and 53 percent hydrophobic amino acids. Another very hydrophobic intrinsic protein is the Folch-Lees protein, which can be isolated from the myelin covering of sciatic nerve; its amino acids are 29 percent hydrophilic and 71 percent hydrophobic. The intrinsic proteins rhodopsin and cytochrome oxidase are both hydrophobic; the amino acids of rhodopsin are 36 percent hydrophilic and 64 percent hydrophobic and those of cytochrome oxidase are 37 percent hydrophilic and 63 percent hydrophobic.

Two interesting intrinsic proteins have recently been characterized that, while they are not excessively hydrophobic in overall composition, have polypeptide-chain regions that are very rich in hydrophobic amino acids. One is cytochrome b_5, a protein isolated from the microsomal membrane of liver cells. Phillip Strittmatter and Lawrence Spatz of the University of Connecticut have shown that this protein, a single polypeptide, is folded at one end into a globular portion that is exposed at the surface of the membrane and is covered predominantly with hydrophilic amino acids. The polypeptide chain continues out of the globular portion into a "tail" of about 60 amino acids, almost all of them hydrophobic. The tail penetrates into the bilayer and serves to anchor the globular and enzymically active portion of the molecule to the membrane.

The second intrinsic protein with an unusual structure is the major glycoprotein found in the membrane of red blood cells. This molecule has been closely studied by Marchesi and various colleagues, first at the National Institutes of Health and more recently at Yale. Again it consists of a single polypeptide chain. One end of the chain, which holds all the carbohydrate associated with the molecule, consists predominantly of hydrophilic amino acids; this end is exposed to the water at the outer surface of the cell membrane. The other end of the chain, which also incorporates hydrophilic amino acids, extends into the watery interior of the red cell. The middle of the chain consists of some 30 amino acids. They are almost exclusively hydrophobic and lie inside the lipid bilayer of the membrane.

Because all the carbohydrate of the glycoprotein molecule is exposed at the outer surface of the red-cell membrane the membrane is asymmetric. Furthermore, labeling studies, using chemical reagents to label "available" proteins, show that the asymmetry in the red-cell membrane extends beyond carbohydrate imbalance. Reagents that cannot penetrate the lipid bilayer of the membrane will label two of the proteins in the membrane of intact red blood cells. One is the glycoprotein; the other is the protein of molecular weight 87,000; only those two proteins are exposed at the outer surface of the membrane. When the red cell is lysed and thereby made leaky to the labeling reagent, however, all the proteins in the membrane are labeled, indicating that the majority of proteins in the red-cell membrane are localized on the membrane's interior surface.

The red-blood-cell membrane is not the only one with an asymmetric organization. Labeling techniques have shown that the mitochondrial inner membrane is organized in a similar fashion. The protein molecules known as headpieces, actually ATPases, are located exclusively on the matrix, or inner, side of the mem-